

Replaced by
Hilde
13 NOV 2000

Claims:

1. A test kit for detecting microbial contaminations in non-sterile products, particularly according to GMP guidelines, including cosmetics and foodstuffs, which test kit comprises at least one DNA fragment comprising the following SEQ IDs and spacers:
 - (a) a forward primer (SEQ ID forward primer);
 - (b) a probe (SEQ ID probe);
 - (c) a reverse primer (SEQ ID reverse primer);
 - (d) optionally a spacer between forward primer and probe,
 - (e) optionally a spacer between probe and reverse primer,
 - (f) optionally a spacer upstream from the forward primer,
 - (g) optionally a spacer downstream from the reverse primer,the SEQ IDs [(SEQ ID forward primer), (SEQ ID probe), and (SEQ ID reverse primer)] also comprising variants wherein one, two or three nucleotides have been substituted, deleted and/or inserted, the variant essentially having the same function as the sequence of the SEQ IDs [(SEQ ID forward primer), (SEQ ID probe), and (SEQ ID reverse primer)],
with probes, the function of binding to DNA, and
with primers, the function of binding to DNA and providing an extendable 3' end for the DNA polymerase,
the spacers comprising 0-40 nucleotides,
the DNA fragment, selected from the group of
 - (i) for *Staphylococcus aureus*
SEQ ID No. 6 as forward primer
SEQ ID No. 7 as probe, and

- (ii) SEQ ID No. 8 as reverse primer
for *Pseudomonas aeruginosa*
SEQ ID No. 9 as forward primer
SEQ ID No. 10 as probe, and
SEQ ID No. 11 as reverse primer
- (iii) for *Escherichia coli*
SEQ ID No. 12 as forward primer
SEQ ID No. 13 as probe, and
SEQ ID No. 14 as reverse primer
- (iv) for *Salmonella ssp.*
SEQ ID No. 15 as forward primer
SEQ ID No. 16 as probe, and
SEQ ID No. 17 as reverse primer
- (v) for bacteria
SEQ ID No. 18 as forward primer
SEQ ID No. 19 as probe, and
SEQ ID No. 20 as reverse primer
- (vi) for enterobacteriaceae
SEQ ID No. 44 as forward primer
SEQ ID No. 46 as probe, and
SEQ ID No. 45 as reverse primer
- (vii) for enterobacteriaceae (16S rRNA)
SEQ ID No. 47 as forward primer
SEQ ID No. 48 as probe, and
SEQ ID No. 49 as reverse primer

or additionally all those sequences which are complementary to the above sequences from SEQ ID No. 6 to 49.

2. A method of detecting microorganisms in products, particularly in drugs or cosmetics, said method comprising the following steps:

- a) use of primers and fluorescence-labelled probes having the appropriate sequences and variations thereof,
 - (i) for *Staphylococcus aureus*
SEQ ID No. 6 as forward primer

- SEQ ID No. 7 as probe, and
 - SEQ ID No. 8 as reverse primer
- (ii) for *Pseudomonas aeruginosa*
 - SEQ ID No. 9 as forward primer
 - SEQ ID No. 10 as probe, and
 - SEQ ID No. 11 as reverse primer
- (iii) for *Escherichia coli*
 - SEQ ID No. 12 as forward primer
 - SEQ ID No. 13 as probe, and
 - SEQ ID No. 14 as reverse primer
- (iv) for *Salmonella ssp.*
 - SEQ ID No. 15 as forward primer
 - SEQ ID No. 16 as probe, and
 - SEQ ID No. 17 as reverse primer
- (v) for bacteria
 - SEQ ID No. 18 as forward primer
 - SEQ ID No. 19 as probe, and
 - SEQ ID No. 20 as reverse primer
- (vi) for enterobacteriaceae
 - SEQ ID No. 44 as forward primer
 - SEQ ID No. 46 as probe, and
 - SEQ ID No. 45 as reverse primer
- (vii) for enterobacteriaceae (16S rRNA)
 - SEQ ID No. 47 as forward primer
 - SEQ ID No. 48 as probe, and
 - SEQ ID No. 49 as reverse primer

or additionally all those sequences which are complementary to the above sequences from SEQ ID No. 6 to 49;

- b) propagating the DNA using PCR, and
- c) irradiating with specific wavelengths exciting the fluorescent dye,
- d) measuring and quantifying the emission of the excited fluorescent dye.

3. The method according to claim 2, wherein the preparation of the probes is based on the TaqMan detection technology.